Promotion of Endometriosis in Mice by Polychlorinated Dibenzo-p-Dioxins, Dibenzofurans, and Biphenyls

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Previous studies showed exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) enhances the development of endometriotic lesions. In this study we examined the effects of other polyhalogenated aromatic hydrocarbons on endometriotic proliferation. B6C3F$_1$ female mice were treated via oral gavage a total of five times, with 3 weeks between each dosing, with 0, 1, 3, or 10 µg 2,3,7,8-TCDD/kg body weight (bw); 3 or 30 mg 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153)/kg bw; 100, 300, or 1000 µg 3,3',4,4',5-pentachlorobiphenyl (PCB 126)/kg bw; 10, 30, or 100 µg 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF)/kg bw; or 2 or 20 mg 1,3,6,8-TCDD/kg at 10 ml/kg bw. Endometriosis was surgically induced during the week of the second dosing. Three weeks following the final dose, the mice were euthanized and endometriotic lesions, whole body, liver, ovaries, uterine horn, and thymus were weighed, and lesion diameters were measured. Lesions, uterine horns, and ovaries were fixed for histopathology and livers were processed for measurement of ethoxyresorufin O-deethylase (EROD) activity. Both 2,3,7,8-TCDD (1 and 3 µg/kg bw) and 4-PeCDF (100 µg/kg bw) significantly enhanced the growth of endometrial lesions. No statistically significant increase in endometriotic lesion size was detected in animals treated with either PCB 126 or with the highest dose of 2,3,7,8-TCDD, possibly due to the effects of histologically observed ovarian toxicity. The nondioxin-like compounds, PCB 153 and 1,3,6,8-TCDD, produced no observable effects on endometriosis. Hepatic EROD activity was significantly induced by 2,3,7,8-TCDD, 4-PeCDF, and PCB 126, but not by PCB 153 or 1,3,6,8-TCDD. The results of this study provide preliminary support for the hypothesis that halogenated aromatic hydrocarbon-promoted endometriosis may be Ah receptor mediated. *Key words*: endometriosis, halogenated aromatic hydrocarbon, polychlorinated biphenyls, polychlorinated dibenzofurans, polychlorinated dibenzo-p-dioxins, structure–activity relationships, TCDD. *Environ Health Perspect* 105:750–755 (1997)

Polyhalogenated aromatic hydrocarbons (PHAHs) are a group of environmental contaminants that include the polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), diphenylethers (PCDEs), biphenyls (PCBs), and naphthalenes (PCNs) (1,2). PHAHs evoke a broad range of toxic and biochemical responses (3,4). One common adverse effect is reproductive toxicity, including reduced fertility, decreased litter size, diminished uterine weight, and altered ovarian functioning in several species (5–7). Recent studies showed an effect on endometriosis in several species following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) (8,9). Endometriosis is the growth of endometrial tissue outside the uterus, often causing infertility and pain (10). A major characteristic of endometriosis is the presence of lesions and hemorrhagic cysts in the peritoneum (11). Proliferation of endometrial lesions is estrogen dependent (12) and often associated with immune dysfunction (13) and it is possibly caused by compounds such as the most potent PHAH, 2,3,7,8-TCDD (14). 2,3,7,8-TCDD induced an increase in the prevalence and severity of endometriosis in rhesus monkeys (8) and provoked an increase in the growth of surgically induced endometriotic lesions in both rats and mice (9) following subchronic

exposure. Because humans are exposed to a broad range of PHAHs, identification of the effects of additional PHAHs on endometriosis is warranted.

Endometriosis is difficult to diagnosis, thus estimates of prevalence vary widely. Many researchers suggest that the prevalence of endometriosis is increasing in the general population (15). While a 10% prevalence rate in the general population has often been accepted (10), estimates are as high as 60–80% among women with infertility or pain in Belgium (16). Researchers suggest that the high incidence rate of endometriosis in Belgian women coincides with elevated concentrations of persistent organochlorine environmental contaminants in this country (17). For example, elevated blood levels of PCBs were discovered in Belgian women who suffer from endometriosis (17). A recent study in Israel has also demonstrated an association between endometriosis and elevated 2,3,7,8-TCDD levels (18).

The mechanism of action by which dioxin induces increased proliferation of endometriosis in recent animal studies (8,9) has not yet been determined. Objectives of this investigation were to determine if PHAH-promotion of endometriosis may be aryl hydrocarbon (Ah) receptor mediated by

evaluating the structural relevance of PHAHs to the proliferation of endometriosis. Therefore, a selection of PHAHs with varying degrees of affinity for the Ah receptor were selected. In addition to 2,3,7,8-TCDD, four other PHAHs were administered: 3,3',4,4',5,-pentachlorobiphenyl (PCB 126), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), 1,3,6,8-tetrachlorodibenzo-p-dioxin (1,3,6,8-TCDD), and 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF). The hypothesis of this study is that induction of endometriosis by PHAHs may be Ah receptor-mediated and will be influenced by structural differences among these chemicals. 2,3,7,8-TCDD and the two additional dioxin-like compounds (PCB 126 and 4-PeCDF) (19) should evoke increased proliferation of endometriotic lesions, while the nondioxin-like chemicals (PCB 153 and 1,3,6,8-TCDD) (19) should not induce increased endometriotic growth.

Materials and Methods

Chemicals. Both dioxin-like and nondioxin-like chemicals were used in addition to 2,3,7,8-TCDD (Radian Corp., Austin, TX) (19). Dioxin-like chemicals used were PCB 126 (Ultra Scientific Chemical Co., North Kingstown, RI) and 4-PeCDF (Accustandard, New Haven, CT), and nondioxin-like chemicals used were PCB 153 (Accustandard) and 1,3,6,8-TCDD (Accustandard.) All chemicals had purities greater than 98%. No 2,3,7,8-TCDD-like contaminants were present in the other chemicals.

Animals. Previous studies using rodent models focused on the effects of 2,3,7,8-TCDD exposure on the promotion of endometriosis in rats and mice (*9*). Because mice showed a greater degree of endometriotic severity (*9*), appeared to lack overt endocrine disruption at the doses studied (9), and demonstrated greater immunosensitivity (*20*) following exposure to 2,3,7,8-TCDD than did rats, the mouse model was chosen for this study. Female B6C3F₁ mice were obtained from Charles River Breeding Laboratories (Raleigh, NC) at 70 days of age, randomly assigned to a treatment group, and acclimated for 1 week prior to dosing. Mice were maintained at the National Health and Environmental Effects Research Laboratory of the U.S. Environmental Protection Agency (EPA). Animal care and treatment were conducted according to established guidelines. All animals were housed in an environment with controlled humidity (40–50%), 12:12-hr light:dark cycle, and constant temperature (20–24°C). Animals received food (Prolab rat, mouse, hamster 3000, Agway, Syracuse, NY) and water *ad libitum*.

Treatment. Chemical doses were assigned based on published toxic equivalency factor (TEF) values (*3*) for all chemicals and solubility considerations for PCB 153 and 1,3,6,8-TCDD. Experiment 1 included doses of 0 (corn oil; Sigma Chemical Co., St. Louis, MO), 1, 3, and 10 μg 2,3,7,8-TCDD/kg bw with 10 animals per group. Experiment 2 included doses of 0, 3, and 30 mg PCB 153/kg bw and 100, 300, and 1000 μg PCB 126/kg bw with 10–12 animals per group. Experiment 3 included doses of 0, 10, 30, and 100 μg 4-PeCDF/kg bw and 2 and 20 mg 1,3,6,8-TCDD/kg bw with 10–12 animals per group. Dosing was administered via oral gavage a total of five times, with 3-week intervals between each dosing as described (*9*). All animals were dosed with a corn oil dosing vehicle at 10 ml/kg bw.

Surgical technique. Because mice have a closed reproductive tract with a bursa-enclosed ovary and an estrous cycle instead of a menstrual cycle, they do not develop endometriosis naturally (*12*). Endometriosis must be induced in these animals through surgical methods performed during the week of the second dosing. The two major steps of the surgical process as described by Vernon and Wilson (*21*) in a rat model and extended to mice by Cummings and Metcalf (*12*) were 1) ablation of the left uterine horn with longitudinal bisection to expose the epithelial cells and 2) suturing of uterine segments onto alternating mesenteric blood vessels in the peritoneal cavity.

Necropsy. At the conclusion of 16 weeks, the animals were euthanized by carbon dioxide asphyxiation followed by exanguination via cardiac puncture. Necropsies were performed in a random order to prevent bias during the measuring of lesion diameters. Lesions, ovaries, uterine horn, liver, and thymus were extracted and weighed. Lesions, ovaries, and uterine horns were fixed for histology, while liver and thymus were frozen on dry ice and stored at -70°C.

EROD assay. Ethoxyresorufin and resorufin were purchased from Molecular Probes (Eugene, OR). Microsomal proteins were prepared (*22*) and quantified (*23*) using bovine serum albumin (BSA) as a standard. The reaction buffer contained 0.1 M KPO₄, 5 mM Mg₂SO₄, and 2 mg BSA/ml at pH 7.5. Liver microsomes were diluted in 0.1 M KPO₄ (100 μl) to provide reaction linearity, added to 0.1 M KPO₄ buffer containing 1.5 nM ethoxyresorufin, and preincubated for 2 min at 37°C. The production of resorufin was started by the addition of 100 μl of β-NADPH (5 mg/ml) and monitored spectrofluorimetrically as described (*22*). A log₁₀ transformation was utilized to normalize ethoxyresorufin O-deethylase (EROD) data.

Histopathology. Microscopic examination of endometriotic lesions and ovaries was performed to characterize histological changes associated with exposure to halogenated aromatic hydrocarbons (HAHs). Animals were selected for histopathology randomly to obtain an unbiased representative sample for review. Histology was prepared by Experimental Pathology Laboratories Inc. (Research Triangle Park, NC) and pathology was performed by John

Seely at PATHCO (Research Triangle Park, NC). Endometriotic lesions were examined for the presence of inflammation and luminal exudate or transudate, while ovaries were examined for both the presence of primary, growing, and antral follicles and the presence of corpora lutea (both active and regressing). Active corpora lutea were defined as newly formed corpora lutea, as well as those that became increasingly eosinophilic and those that appeared foamy. Regressive corpora lutea were characterized by degeneration, necrosis, and fibrous tissue proliferation.

Statistical analysis. Primary statistical analysis of endometriotic lesion diameters and secondary analyses of lesion, ovarian, uterine, and thymus weights from all chemical treatment groups were performed using the Dunnett's test and a level of probability of statistical significance of $p<0.05$. Means with standard deviations (SD) were determined for all dose groups for body, liver, ovarian, uterine, and lesion weights and lesion diameters. For EROD activity, the statistical intergroup comparisons were determined using a one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD). The levels of probability of statistical significance for EROD data are $p<0.01$.

Results

EROD activity is a marker for CYP1A1-dependent enzyme induction by 2,3,7,8-TCDD and related compounds (*19*). In this study, constitutive EROD activity in control animals for three separate experiments were similar (Table 1). A statistically

Table 1. Mean parameter values and standard deviations

Chemical	Dose (per kilogram body weight)	EROD activity (pmoles/min/ mg protein)	Lesion diameter (mm)	Lesion weight (mg)	Ovarian weight[a] (mg)
Control 1	0 μg	114 ± 40	5.46 ± 1.27	22.50 ± 13.0	8.51 ± 0.81
2,3,7,8-TCDD	1 μg	201 ± 100	7.27 ± 1.42*	109.20 ± 63.60*	9.11 ± 2.18
	3 μg	657 ± 378**	6.96 ± 0.77*	94.50 ± 43.30*	9.30 ± 1.45
	10 μg	844 ± 386**	6.12 ± 0.76	61.70 ± 26.70*	7.03 ± 2.05
Control 2	0 mg	71 ± 27	5.71 ± 1.43	71.30 ± 51.50	8.38 ± 2.98
PCB 153	3 mg	116 ± 51	5.74 ± 0.90	59.50 ± 26.20	10.22 ± 1.14
	30 mg	91 ± 32	5.84 ± 0.52	64.10 ± 18.90	13.49 ± 10.57
PCB 126	100 μg	805 ± 408**	6.25 ± 1.03	65.70 ± 28.90	10.68 ± 1.97
	300 μg	1576 ± 420**	6.83 ± 1.46	101.00 ± 55.80	9.91 ± 1.56
	1000 μg	1827 ± 439**	6.61 ± 1.75	91.90 ± 57.90	9.14 ± 2.25
Control 3	0 mg	110 ± 34	6.05 ± 0.86	71.00 ± 31.70	10.78 ± 1.81
1,3,6,8-TCDD	2 mg	84 ± 23	5.98 ± 0.91	63.30 ± 27.80	9.17 ± 1.72
	20 mg	159 ± 49	5.96 ± 0.82	61.00 ± 29.40	10.38 ± 1.56
4-PeCDF	10 μg	676 ± 293**	6.05 ± 0.54	70.00 ± 22.00	10.42 ± 2.38
	30 μg	1303 ± 527**	6.26 ± 1.27	84.20 ± 46.40	9.58 ± 1.72
	100 μg	1516 ± 782**	7.32 ± 1.29*	114.30 ± 58.50	9.08 ± 1.09

Abbreviations: 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PCB 153, 2,4,5,2′,4′,5′-hexachlorobiphenyl; PCB 126, 3,4,5,3′,4′-pentachlorobiphenyl; 4-PeCDF, 2,3,4,7,8-pentachlorodibenzofuran, 1,3,6,8-TCDD, 1,3,6,8-tetrachlorodibenzo-*p*-dioxin.
[a]Right ovary of intact uterine horn.
*$p<0.05$; **$p<0.01$.

significant ($p<0.01$) dose-dependent increase in EROD activity in mice treated with increasing doses of 2,3,7,8-TCDD, PCB 126, or 4-PeCDF was observed; in contrast, mice treated with 1,3,6,8-TCDD or PCB 153 had EROD activities similar to control groups (Table 1).

Previous studies in a rodent model for 2,3,7,8-TCDD-promoted endometriosis focused on lesion diameter as an indicator of 2,3,7,8-TCDD-induced responses (9). Examination of lesion diameter in three separate control groups in this study indicated similar values with no statistical differences (Table 1). Treatment of animals with 1 or 3 µg 2,3,7,8-TCDD/kg bw resulted in a statistically significant increase in lesion diameter ($p<0.05$). Although the diameter was increased relative to controls at 10 µg 2,3,7,8-TCDD/kg bw, it was not statistically significant (Table 1). An increase in lesion diameter with dose was observed in animals treated with 4-PeCDF, although a statistically significant increase was only observed in animals treated with 100 µg 4-PeCDF/kg bw (Table 1). Animals treated with PCB 126 resulted in an apparent increase in lesion diameter; however, this increase was not statistically significant compared to control animals (Table 1). Analysis of lesion diameter values for animals treated with PCB 153 or 1,3,6,8-TCDD resulted in lesion diameter values similar to control animals in all dose groups (Table 1).

Endometriotic lesion weight was used as an additional marker to examine HAH-promoted endometriosis. Examination of lesion weights in three separate control groups revealed high variability. Lesion weights for control animals in experiment 1 were significantly different from control animals in experiments 2 and 3, which may contribute to the variability in results among dose and chemical groups (Table 1). Treatment of animals with 1, 3, or 10 µg 2,3,7,8-TCDD/kg bw resulted in a dose-dependent decrease in endometriotic lesion weight. However, endometriotic lesion weights in all dose groups of 2,3,7,8-TCDD-treated mice were significantly elevated compared to control animals in experiment 1 (Table 1). Lesion weights of animals treated with 1 or 3 µg 2,3,7,8-TCDD/kg bw also appear elevated when compared to control animals of experiments 2 or 3. Elevated endometriotic lesion weights were not observed in the lowest treatment groups for 4-PeCDF or PCB 126 when compared to controls, although an apparent, but nonsignificant dose-dependent increase in lesion weight was observed in animals treated with 4-PeCDF/kg bw or 100 or 300 µg PCB 126/kg bw. Lesion

weight values for the highest dose of PCB 126 (1000 µg/kg bw) decreased nonsignificantly from 300 µg/kg bw. High variability was present in all treatment groups, especially in lesion weight data of animals treated with PCB 126 or 4-PeCDF. Analysis of lesion weights in mice treated with PCB 153 or 1,3,6,8-TCDD resulted in endometriotic lesion weights similar to control animals.

Another tissue examined in this study to assess the effects of HAH exposure on endometriosis was the ovary. Because body weight values did not vary significantly across chemical classes or doses, similar results were observed between crude ovarian weights (Table 1) and ovarian weight/bw ratios (data not shown). Ovarian weights from ablated uterine horns appeared slightly lower than the ovarian weights from intact uterine horns. However, trends in both sets of ovarian weights are consistent within most dose groups. Therefore, the crude ovarian weights from intact uterine horns (Table 1) were used as markers to describe the dose-dependent effects of administered compounds on ovarian weights. Although no significant differences in ovarian weight were found in comparisons of treated to control animals, possibly due to high data variability, examination of ovarian weights from animals treated with 1 or 3 µg 2,3,7,8-TCDD/kg bw revealed a trend toward an increase in ovarian weights, which was followed by an apparent decrease in ovarian weight in animals treated with 10 µg 2,3,7,8-TCDD/kg bw (Table 1). Animals treated with PCB 153 exhibited an apparent but nonsignificant increase in ovarian weight with increasing dose (Table 1). In contrast, mice treated with PCB 126 or 4-PeCDF

showed an apparent decrease in ovarian weight with increasing dose (Table 1). Furthermore, analysis of ovarian weights from mice treated with 1,3,6,8-TCDD showed ovarian weights similar to control animals at all dose levels (Table 1). A microscopic examination of endometriotic lesions and ovarian tissue was used to characterize histological changes associated with exposure. In all endometrial lesions examined, endometrial epithelium, endometrial glands with stroma, and the myometrium were present, but these structures varied in thickness and prominence, without relation to treatment. The presence of luminal exudate or transudate was more severe in lesions characterized as nonstandard than in those characterized as standard, but no significant dose-dependent distribution of severity was noticed because incidence rates of nonstandard lesions, such as discolored or hardened lesions, were consistent across dose groups and chemical classes. Inflammation of the endometriotic uterine segments was consistent across all animals and, in most instances, appeared acute (associated with polymorphonuclear cells.) Examination of ovarian tissue revealed the absence of active corpora lutea in animals only from the 10 µg 2,3,7,8-TCDD (in two of three animals examined), 100 µg PCB 126 (one of three animals), and 1000 µg PCB 126/kg bw (two of three animals) treatment groups. In addition, animals with the highest number of regressive corpora lutea compared to total corpora lutea (both active and regressive) were animals treated with 10 µg 2,3,7,8-TCDD (53.3%), 100 µg PCB 126 (60%), or 1000 µg PCB 126/kg bw (75%). In contrast, only 26.7% of the total corpora lutea in control animals, was characterized as regressive.

Table 2. Mean parameter values and standard deviations

Chemical	Dose (per kilogram body weight)	Uterine horn weight[a] (g)	Thymus weight (g)	Liver weight (g)
Control 1	0 µg	0.069 ± 0.016	0.031 ± 0.012	1.247 ± 0.107
2,3,7,8-TCDD	1 µg	0.060 ± 0.020	0.035 ± 0.014	1.359 ± 0.148
	3 µg	0.074 ± 0.029	0.048 ± 0.022	1.323 ± 0.115
	10 µg	0.058 ± 0.020	0.031 ± 0.013	1.581 ± 0.159
Control 2	0 mg	0.050 ± 0.023	0.033 ± 0.004	1.238 ± 0.140
PCB 153	3 mg	0.057 ± 0.019	0.035 ± 0.010	1.291 ± 0.166
	30 mg	0.058 ± 0.020	0.042 ± 0.011	1.306 ± 0.146
PCB 126	100 µg	0.049 ± 0.008	0.043 ± 0.012	1.410 ± 0.160
	300 µg	0.058 ± 0.015	0.031 ± 0.012	1.367 ± 0.151
	1000 µg	0.053 ± 0.011	0.028 ± 0.008	1.521 ± 0.128
Control 3	0 mg	0.061 ± 0.017	0.036 ± 0.005	1.329 ± 0.081
1,3,6,8-TCDD	2 mg	0.053 ± 0.014	0.039 ± 0.009	1.197 ± 0.087
	20 mg	0.061 ± 0.016	0.041 ± 0.007	1.300 ± 0.182
4-PeCDF	10 µg	0.063 ± 0.015	0.036 ± 0.007	1.359 ± 0.131
	30 µg	0.060 ± 0.029	0.027 ± 0.008*	1.440 ± 0.310*
	100 µg	0.052 ± 0.023	0.026 ± 0.006*	1.467 ± 0.211*

Abbreviations: 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PCB 153, 2,4,5,2′,4′,5′-hexachlorobiphenyl; PCB 126, 3,4,5,3′,4′-pentachlorobiphenyl; 4-PeCDF, 2,3,4,7,8-pentachlorodibenzofuran, 1,3,6,8-TCDD, 1,3,6,8-tetrachlorodibenzo-*p*-dioxin.
[a]Intact uterine horn.
*$p<0.05$.

The absence of active corpora lutea and the high percentages of regressive corpora lutea in these dose groups indicate either a direct or indirect chemically induced atrophic effect on the ovaries in these animals.

The final parameters measured in the study were uterine, thymus, and liver weights. Uterine weights of all treated animals were not statistically different from values of control animals, and analysis of uterine weights for three separate control experiments revealed no statistical differences (Table 2). There was an apparent nonsignificant increase in uterine weights of mice treated with 3 μg 2,3,7,8-TCDD/kg bw, which was followed by an apparent decrease in uterine weights in the highest dose group, 10 μg 2,3,7,8-TCDD/kg bw. These effects on uterine weights were similar to those observed in animals treated with PCB 126. A trend toward a slight decrease in uterine weights with increasing dose of 4-PeCDF was observed. Treatment of animals with all doses of PCB 153 resulted in a slight, nonsignificant dose-dependent increase in uterine weights as compared to controls. However, all animals treated with 1,3,6,8-TCDD had uterine weights similar to control animals. In contrast, a dose-dependent decrease in thymus weights with increasing dose of 4-PeCDF or PCB 126 was observed (Table 2). Thymus weights of animals treated with 30 or 100 μg 4-PeCDF/kg bw were significantly less than control thymus weights ($p<0.05$), while the suggested decrease in thymus weights in animals treated with PCB 126 was nonsignificant. Finally, treatment of animals with 2,3,7,8-TCDD, PCB 126, or 4-PeCDF resulted in an apparent increase in liver weight with increasing dose, although this was only significant in animals treated with the middle and high doses of 4-PeCDF (Table 2). In contrast, exposure of animals to PCB 153 or 1,3,6,8-TCDD resulted in liver weights in all dose groups similar to controls.

Discussion

The promotion of endometriosis only by PHAHs with high binding affinity to the Ah receptor is consistent with the hypothesis that PHAH promotion of endometriosis is Ah receptor mediated. Due to variability in these results from study limitations such as individual animal variability, bioassay insensitivity, and gross invasiveness of the surgical procedures, conclusions about the mechanism of action by which HAHs promote endometriosis are only preliminary. Follow-up studies should attempt to refine the surgical model to more accurately assess the effects of HAHs on endometriosis.

As in previous studies that focused on lesion diameter as the primary endpoint to assess the influence of 2,3,7,8-TCDD on the promotion of surgically induced endometriosis (9), this study evaluated the effects of 2,3,7,8-TCDD, PCB 126, PCB 153, 1,3,6,8-TCDD, and 4-PeCDF on endometriotic lesion diameter (Table 1). The mechanism by which PHAHs such as 2,3,7,8-TCDD, PCB 126, or 4-PeCDF increase lesion diameter is unknown, but it appears to be Ah receptor mediated, as is true for all other well-characterized 2,3,7,8-TCDD-induced responses (24).

Structure binding relationships (SBRs), based on affinity of ligand binding for the Ah receptor, are often indirectly assessed by induction of arylhydrocarbon hydroxylase (AHH), EROD, and other enzyme activities, and are described by structure–activity relationships (SARs). Previous studies showed a correlation between endpoints, such as CYP1A1 induction, and affinity for the Ah receptor expressed through SARs (25). For example, in this study 2,3,7,8-TCDD, PCB 126, and 4-PeCDF, the congeners that increased endometriotic lesion diameters (statistically significant only in animals treated with 2,3,7,8-TCDD or 4-PeCDF), have the strongest binding affinities for the Ah receptor (1,26,27). In contrast, compounds such as 1,3,6,8-TCDD and PCB 153, which did not induce any increases in endometriotic lesion diameter, have weak affinities towards the Ah receptor (27,28).

Endometriotic lesion weights were also measured for secondary analysis of changes in endometriotic lesion sizes due to HAH exposure (Table 1). Increases in lesion weight, like increases in lesion diameter, occured in animals exposed to chemicals with the highest affinities for the Ah receptor (statistically significant increases induced by 2,3,7,8-TCDD and nonsignificant increases induced by PCB 126 and 4-PeCDF) and not in animals exposed to chemicals with significantly lower binding affinities (PCB 153 and 1,3,6,8-TCDD). This supports the hypothesis that promotion of endometriosis by HAHs may be Ah receptor mediated.

This study also revealed changes in ovarian weight and differences in ovarian histopathological evaluation based on chemical and dose (Table 1). As with other endpoints, chemicals with greater binding affinities for the Ah receptor evoked greater toxic responses such as decreases in ovarian weights. Ovarian histological examination of animals treated with 1 or 3 μg 2,3,7,8-TCDD/kg bw, PCB 153, 1,3,6,8-TCDD, or 4-PeCDF was consistent with ovarian weights for these animals and indicated no presence of ovarian atrophy. The absence of corpora lutea and the high percentages of regressive corpora lutea in animals treated

with 10 μg 2,3,7,8-TCDD, or 100 or 1000 μg PCB 126/kg bw supports the hypothesis that ovarian atrophy may have occurred in animals treated with high doses of chemicals with strong binding affinities for the Ah receptor. The lack of ovarian atrophy observed in the 4-PeCDF-treated animals may be due to the low concentration of 4-PeCDF available to extrahepatic tissues due to its sequestration in the liver (29). Still, the results of the histological evaluations are preliminary because only a small, randomly selected representative sample from each dose group was examined.

Ovarian atrophy at high doses of 2,3,7,8-TCDD and PCB 126 is consistent with the results of decreased lesion diameters and weights observed in animals administered high doses of these compounds compared to the resulting lesion diameters and weights in animals administered lower doses. High doses of PHAHs may cause antiestrogenic effects, leading to either indirect or direct ovarian toxicity or atrophy (30). The resulting decrease in the promotion of endometriosis (observable in the variability in lesion diameter and lesion weight values at the high dose levels of 2,3,7,8-TCDD and PCB 126) is consistent with the requirement for estrogen to promote lesion growth (31). Thus, Ah receptor binding may correlate with ovarian atrophy, as well as lesion diameter and lesion weight. Future studies examining endometrial promotion should use lower dose levels of 2,3,7,8-TCDD and PCB 126 to avoid adverse effects on the ovary.

Several additional endpoints were measured in this study to assess the adverse effects of PHAH exposure. Resulting antiestrogenic effects from HAH exposure could also be mediated by an indirect route via a decrease in the concentration of circulating estrogens (32). This can also lead to a decrease in uterine weights (33). Analysis of uterine weights in this study revealed a correlation between changes in uterine weights and binding affinities for the Ah receptor. Chemicals that bind strongly to the Ah receptor (2,3,7,8-TCDD, PCB 126, and 4-PeCDF) caused decreases in uterine weights at high doses, while chemicals that bind weakly to the Ah receptor (PCB 153 and 1,3,6,8-TCDD) evoked no apparent decreases in uterine weights when compared to controls. Also, even though changes in uterine weights were not statistically significant, they appeared to correlate with changes in ovarian weights and ovarian histopathological evaluations, supporting the theory of ovarian atrophy in animals treated with the highest doses of 2,3,7,8-TCDD or PCB 126. Therefore, a relationship appears to exist between structure–activity and binding

relationships and antiestrogenic effects, such as decreases in uterine weights.

Another significant effect often associated with 2,3,7,8-TCDD exposure is thymic atrophy, shown by previous studies to be Ah receptor mediated (25). Originally, this study design was for levels of chemical exposure to produce no overt toxicity. However, analysis of thymic atrophy in this study revealed decreases in thymus weights only at high doses of chemicals with high binding affinities for the Ah receptor (2,3,7,8-TCDD, PCB 126, and 4-PeCDF); this was only statistically significant in animals treated with either of the two highest doses of 4-PeCDF. Therefore, the mechanism of thymic atrophy correlates with the structural relationships of PHAHs observed in mice with endometriosis.

Hepatotoxicity is a common response in mice following exposure to PHAHs (32) and often results in increased liver weights (34). In this study, nonsignificant increased liver weights with increasing dose were apparent only in animals treated with 2,3,7,8-TCDD or PCB 126, while statistically significant increases were apparent in animals treated with 4-PeCDF. A greater hepatotoxic response may have been observed in animals treated with 4-PeCDF than in animals treated with 2,3,7,8-TCDD or PCB 126 because 4-PeCDF is sequestered in the liver (29). Because these chemicals bind with great affinity to the Ah receptor, increases in liver weight and hepatotoxicity appear to correlate with binding affinities for the Ah receptor.

In addition to induction of toxic responses such as thymic atrophy and hepatotoxicity, PHAHs have been identified as microsomal monooxygenase inducers (35), frequently inducing cytochrome P450 isozymes (36). CYP4501A1 enzyme induction is often measured by AHH or EROD activity. Increased enzyme activity coincides with increased gene expression and PHAH exposure (37). Therefore, EROD activity was measured in this study as an indicator of PHAH-induced effects and to interpret information about endometrial growth. Based solely on analysis of EROD activities (Table 1) for animals treated with PCB 126 versus animals treated with 2,3,7,8-TCDD, lesion size of animals treated with the 100 µg PCB 126/kg bw should be comparable to lesions in animals treated with 10 µg 2,3,7,8-TCDD/kg bw. Thus, lesion diameters of animals treated with PCB 126 should have decreased with increasing dose as did lesion diameters in animals treated with the highest dose of 2,3,7,8-TCDD when compared to lower doses. This effect is most probably the result of ovarian atrophy as seen histologically. Even though the EROD

values of animals treated with the highest dose of 4-PeCDF were comparable to EROD values in animals treated with 300 µg PCB 126/kg bw, no ovarian atrophy was observed in these animals, possibly because of the lack of availability of this chemical to extrahepatic tissue due to its extensive sequestration in the liver (29). Thus, lesion diameters continued to increase because no ovarian atrophy occurred and circulating estrogen levels were probably normal.

In conclusion, this study was an analysis of the influence of the structural relevance of PHAHs on the proliferation of surgically induced endometriotic lesions in B6C3F$_1$ female mice. Analysis of all the parameters measured, especially lesion diameter, suggests that the mechanism of PHAH-promoted endometriosis may be Ah receptor mediated, with structure–activity and binding relationships influencing the degree of endometriotic proliferation.

Some endpoints in this study, such as endometriotic lesion diameter, did not correlate directly with dose level or hepatic EROD induction because of influences of additional responses such as hormonal interactions and possible antiestrogenic effects. Chemicals exerting antiestrogenic effects at high doses can induce ovarian atrophy, which in turn may decrease circulating estrogen levels and proliferation of endometriosis. In general, the responses in lesion diameter, lesion weight, ovarian weight, uterine weight, and thymus weight correlate with structure binding relationships of the administered PHAHs. Specifically, analysis of the primary endpoint measured, endometriotic lesion diameter, demonstrates a dose-dependent increase in size following administration of chemicals with strong binding affinities for the Ah receptor, when the effects of ovarian atrophy on lesion diameter are controlled. Therefore, the results of this study provide preliminary support for the hypothesis that PHAH promotion of endometriosis may be Ah receptor mediated.

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